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#13	Search #2 NOT (#3 or #4) Field: Title/Abstract,	14:53:11	<u>99</u>
,	Limits: Publication Date to 2001/04/04		
<u>#12</u>	Search #2 NOT (#3 or #4) Field: Title/Abstract	14:26:46	214
#9	Search #3 NOT #4 Field: Title/Abstract	14:26:21	8
#4	Search #2 AND (culture or media or cystein* or sulfat*) AND (toxin or pertussis or PT) Field: Title/Abstract	14:23:01	<u>46</u>
#3	Search #2 AND (culture or media or cystein* or sulfat*) AND (toxin or pertussis or PT)	14:18:19	<u>54</u>
#2	Related Articles for PubMed (Select 12595447)	14:17:31	<u> 268</u>
<u>#1</u>	Search 12595447	14:17:28	1

Clear History

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Nov 8 2004 18:23:56

FILE 'HOME' ENTERED AT 18:08:10 ON 15 NOV 2004

L6

L1 2242 (BARIUM OR BACL## OR SULFATE OR SO4## OR CYSTEINE) AND (BACTER? OR PATHOGEN? OR BORDETELLA OR SHIGELLA OR STAPH?) (P) #####TOXIN

706 L5 AND (BACTER? OR CLOSTRID? OR BORDETELLA OR SHIGELLA OR STAPH ?)

(FILE 'HOME' ENTERED AT 18:08:10 ON 15 NOV 2004)

	FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE' ENTERED AT 18:09:47 ON 15 NOV 2004
L1	2242 S (BARIUM OR BACL## OR SULFATE OR SO4## OR CYSTEINE) AND (BACTE
L2	533 S L1 AND (CYSTEINE OR CYS?) (P) TOXIN
L3	37 S L2 AND (SULFATE OR METABOL?) (S) (CYSTEINE OR TOXIN)
L4	24 DUP REM L3 (13 DUPLICATES REMOVED)
L5	765 S L1 AND (SULFATE OR SO4##) (P) TOXIN
L6	706 S L5 AND (BACTER? OR CLOSTRID? OR BORDETELLA OR SHIGELLA OR S
L7	440 S L1 AND (SULFATE OR SO4##) (S) TOXIN
L8	420 S L7 NOT L2
L9	22 S L8 AND (INHIBIT (S) TOXIN)
L10	27 S L7 AND (INHIBIT (S) TOXIN)
L11	31 S L6 AND (INHIBIT (S) TOXIN)
L12	19 DUP REM L11 (12 DUPLICATES REMOVED)

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DUPLICATE 2
                      MEDLINE on STN
     ANSWER 3 OF 24
AN
     2003084092
                   MEDLINE
DN
     PubMed ID: 12595447
    Reduced glutathione is required for pertussis toxin secretion by
ТT
    Bordetella pertussis.
     Stenson Trevor H; Patton Angela K; Weiss Alison A
AIJ
     Department of Molecular Genetics, Biochemistry, and Microbiology,
CS
     University of Cincinnati, Cincinnati, Ohio 45267-0524, USA.
     R01 AI23695 (NIAID)
NC
     Infection and immunity, (2003 Mar) 71 (3) 1316-20.
SO
     Journal code: 0246127. ISSN: 0019-9567.
     United States
CY
     Journal; Article; (JOURNAL ARTICLE)
DT
     English
LΑ
FS
     Priority Journals
     200303
EM
     Entered STN: 20030222
     Last Updated on STN: 20030321
     Entered Medline: 20030320
     The abilities of cysteine-containing compounds to support growth
AB
     of Bordetella pertussis and influence pertussis toxin
     transcription, assembly, and secretion were examined. Cysteine
     is an essential amino acid for B. pertussis and must be present for
     protein synthesis and bacterial growth. However,
     cysteine can be metabolized to sulfate, and
     high concentrations of sulfate can selectively inhibit
     transcription of the virulence factors, including pertussis toxin
     , via the BvgAS two-component regulatory system in a process called
     modulation. In addition, pertussis toxin possesses several
     disulfide bonds, and the cysteine-containing compound
     glutathione can influence oxidation-reduction reactions and perhaps
     disulfide bond formation. Bacterial growth was not observed in
     the absence of a source of cysteine. Oxidized glutathione, as a
     sole source of cysteine, also did not support bacterial
     growth. Cysteine, cystine, and reduced glutathione
     did support bacterial growth, and none of these compounds caused
     modulation at the concentrations tested. Similar amounts of periplasmic
     pertussis toxin were detected regardless of the source of
     cysteine; however, in the absence of reduced glutathione,
     pertussis toxin was not efficiently secreted. Addition of the
     reducing agent dithiothreitol was unable to compensate for the lack of
     reduced glutathione and did not promote secretion of pertussis
     toxin. These results suggest that reduced glutathione does not
     affect the accumulation of assembled active pertussis toxin in
     the periplasm but plays a role in efficient pertussis toxin
     secretion by the bacterium.
     ANSWER 4 OF 24 CAPLUS COPYRIGHT 2004 ACS on STN
T.4
     2001:747833 CAPLUS
AN
     135:302952
DN
     Improved method for the production of bacterial toxins
TI
    Blake, Milan S.; Bogdan, John A., Jr.; Nazario-Larrieu, Javier
     Baxter International Inc., USA; Baxter Healthcare S.A.
PA
     PCT Int. Appl., 46 pp.
SO
     CODEN: PIXXD2
DT
     Patent
LA
     English
FAN.CNT 1
                                          APPLICATION NO.
                                                                   DATE
                         KIND
                                DATE
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PATENT NO.

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20010404
                                           WO 2001-US10938
    WO 2001074862
                         A2
                                20011011
PΙ
                                20021003
     WO 2001074862
                         A3
         W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
             HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
             LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO,
             RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN,
             YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
         RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
             DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,
             BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
                                          US 2001-825770
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                                20020523
                         Α1
     US 2002061555
                         B2
                                20040203
     US 6686180
     US 2002165344
                                20021107
                                            US 2001-825769
                                                                   20010404
                         A1
                                          EP 2001-926612
                         A2
                                20030102
     EP 1268531
         R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
             IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
                                           JP 2001-572551
                                                                   20010404
                                20031028
     JP 2003531586
                         T2
PRAI US 2000-194478P
                          P
                                20000404
     US 2000-194482P
                         Ρ
                                20000404
     WO 2001-US10938
                         W
                                20010404
     Methods and compns. are provided for the enhanced production of
AB
     bacterial toxins in large-scale cultures. Specifically, methods
     and compns. for reducing bacterial toxin expression
     inhibitors are provided including, but not limited to, addition of
     toxin expression inhibitor binding compds., culture media having
     reduced concns. of toxin inhibitor metabolic
     precursors and genetically modified toxigenic bacteria lacking
     enzymes required to metabolize the toxin inhibitor
     metabolic precursors.
                                                        DUPLICATE 3
     ANSWER 5 OF 24
                        MEDLINE on STN
L4
     2001551434
                    MEDLINE
AN
     PubMed ID: 11598055
DN
     Bordetella pertussis autoregulates pertussis toxin
TI
     production through the metabolism of cysteine.
     Bogdan J A; Nazario-Larrieu J; Sarwar J; Alexander P; Blake M S
ΑU
     Baxter Healthcare Corporation, Columbia, Maryland 21046-2358, USA..
CS
     John Bogdan@Baxter.com
     Infection and immunity, (2001 Nov) 69 (11) 6823-30.
SO
     Journal code: 0246127. ISSN: 0019-9567.
CY
     United States
     Journal; Article; (JOURNAL ARTICLE)
DT
LА
     English
FS
     Priority Journals
     200112
ΕM
     Entered STN: 20011015
ED
     Last Updated on STN: 20021218
     Entered Medline: 20011205
     Pertussis toxin (Ptx) expression and secretion in
AΒ
     Bordetella pertussis are regulated by a two-component signal
     transduction system encoded by the bvg regulatory locus. However, it is
     not known whether the metabolic pathways and growth state of the
     bacterium influence synthesis and secretion of Ptx and other
     virulence factors. We have observed a reduction in the concentration of
     Ptx per optical density unit midway in fermentation. Studies were
     conducted to identify possible factors causing this reduction and to
     develop culture conditions that optimize Ptx expression. Medium
     reconstitution experiments demonstrated that spent medium and a fraction
     of this medium containing components with a molecular weight of <3,000
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inhibited the production of Ptx. A complete flux analysis of the

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intermediate metabolism of B. pertussis revealed that the sulfur-containing amino acids methionine and cysteine and the organic acid pyruvate accumulated in the media. In fermentation, a large amount of internal sulfate (SO4(2-)) was observed in early stage growth, followed by a rapid decrease as the cells entered into logarithmic growth. This loss was later followed by the accumulation of large quantities of **SO4**(2-) into the media in late-stage fermentation. Release of SO4(2-) into the media by the cells signaled the decoupling of cell growth and Ptx production. Under conditions that limited cysteine, a fivefold increase in Ptx production was observed. Addition of barium chloride (BaCl2) to the culture further increased Ptx yield. Our results suggest that B. pertussis is capable of autoregulating the activity of the bvg regulon through its metabolism of cysteine. Reduction of the amount of cysteine in the media results in prolonged vir expression due to the absence of the negative inhibitor SO4(2-). Therefore, the combined presence and metabolism of cysteine may be an important mechanism in the pathogenesis of B. pertussis.

- ANSWER 8 OF 24 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
- 2002:176480 BIOSIS AN
- PREV200200176480
- Identification and characterization of a cysteine desulfinase ТΤ gene in Bordetella pertussis.
- Yuan, W. [Reprint author]; Bogdan, J. A. [Reprint author]; Blake, M. S. ΑU [Reprint author]
- Baxter Healthcare Corporation, Columbia, MD, USA CS
- Abstracts of the General Meeting of the American Society for Microbiology, SO (2001) Vol. 101, pp. 87. print. Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology.
 - ISSN: 1060-2011.
- Conference; (Meeting) DT Conference; Abstract; (Meeting Abstract)
- T.A
- Entered STN: 6 Mar 2002
 - Last Updated on STN: 6 Mar 2002
- Many studies have shown that sulfate ions inhibit the production AB of pertussis toxin (Ptx). We have shown that sulfur containing amino acids, methionine and cysteine, accumulate during fermentation in the late exponential phase of bacterial growth in concert with the appearance of sulfate anion in the media. Ptx expression begins to wane approximately at the same time as measurable sulfate anion can be detected. Our hypothesis is that the accumulation of sulfate anion acts as a natural negative feedback inhibitor of Ptx expression. An NIFS-like protein of E. coli has been cloned and reported to have cysteine desulfinase activity, removing the sulfate ion from cysteine. We have identified a similar cysteine desulfinase (dsf) gene on a 1.2 Kb DNA fragment from a B. pertussis genomic library. The DNA sequence of the region showed an ORF having a striking sequence homology at the translated protein level with the dsf gene of E. coli. Analysis by Southern blotting, using the full-length gene as the probe, demonstrated that only a single copy was present in the genome of three different B. pertussis strains. To determine the expression pattern of the desulfinase gene in our B. pertussis strain, we performed RT-PCR on total RNA extracted from the cell pellets harvested at different time points during fermentation.

These studies showed that 'cdsf' transcription increased at 10 hours during fermentation, which correlated well with the observed increase of sulfate in the media.

- L4 ANSWER 9 OF 24 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
- AN 2002:176481 BIOSIS
- DN PREV200200176481
- TI Bordetella pertussis auto-regulates pertussis toxin production through the metabolic conversion of L-cysteine to pyruvic acid and sulfate.
- AU Bogdan, J. A. [Reprint author]; Yuan, W. [Reprint author]; Sarwar, J. [Reprint author]; Alexander, P. [Reprint author]; Blake, M. S. [Reprint author]
- CS Baxter Healthcare Corporation, Columbia, MD, USA
- SO Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 87. print.

 Meeting Info.: 101st General Meeting of the American Society for

Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society for Microbiology.

ISSN: 1060-2011.

DT Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)

LA English

ED Entered STN: 6 Mar 2002

Last Updated on STN: 6 Mar 2002

Pertussis toxin (Ptx) synthesis and secretion in AB Bordetella pertussis is regulated via a two component signal transduction system encoded by the bvg regulatory locus. BvgS, is a sensory protein in the outer membrane that regulates the expression and secretion of Ptx in response to environmental stimuli such as MgSO4, nicotinic acid and growth at low temperatures. Mutations in BvgS, the RNA polymerase alpha subunit and the proteins of the secretory apparatus directly influence Ptx synthesis and secretion. However, it is not known whether the metabolic pathways and growth-state of the bacteria influences synthesis and secretion of Ptx and other virulence factors. Previously, we have shown that sulfate (SO4) appears in the media in B. pertussis fermentation and in turn acts as a negative feedback inhibitor of Ptx expression. Cysteine desulfurase is a metabolic enzyme that converts L-cysteine into pyruvic acid and so4. To determine whether this metabolic pathway was involved in Ptx production, experiments were designed that limited the amount of L-cysteine in the media. Under these conditions, we observed a delay in the release of internal SO4 into media and an increase in the amount of Ptx. Cellular extracts were analyzed for the appearance of the Vra-b protein, a marker for the Bvgphase. In fermentations using limiting amounts of L-cysteine, the Vra-b protein was absent. In fermentation runs using standard media, the Vra-b protein appeared following the appearance of \$04. We have cloned and sequenced the B. pertussis homologue to the E. coli cysteine desulfurase gene. Preliminary results suggest that that there is an increase in cysteine desulfurase transcription before the appearance of SO4 in the media.

- L4 ANSWER 10 OF 24 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
- AN 2000347465 EMBASE
- Toxins, butyric acid, and other short-chain fatty acids are coordinately expressed and down-regulated by cysteine in Clostridium difficile.
- AU Karlsson S.; Lindberg A.; Norin E.; Burman L.G.; Akerlund T.

- T. Akerlund, Department of Bacteriology, Swedish Inst. Infect. Dis. Contr., S-171 82 Solna, Sweden. Thomas.Akerlund@smi.ki.se
- Infection and Immunity, (2000) 68/10 (5881-5888). SO

ISSN: 0019-9567 CODEN: INFIBR

- United States CY
- Journal; Article DТ
- Microbiology FS 004
- English LΑ
- SL
- English It was recently found that a mixture of nine amino acids down-regulate AB Clostridium difficile toxin production when added to peptone yeast extract (PY) cultures of strain VPI 10463 (S. Karlsson, L. G. Burman, and T. Akkerlund, Microbiology 145:1683-1693, 1999). In the present study, seven of these amino acids were found to exhibit a moderate suppression of toxin production, whereas proline and particularly cysteine had the greatest impact, on both reference strains (n = 6) and clinical isolates (n = 28) of C. difficile (>99%) suppression by cysteine in the highest toxin-producing strain). Also, cysteine derivatives such as acetylcysteine, glutathione, and cystine effectively down-regulated toxin expression. An impact of both cysteine and cystine but not of thioglycolate on toxin yield indicated that toxin expression was not regulated by the oxidation-reduction potential. Several metabolic pathways, including butyric acid and butanol production, were coinduced with the toxins in PY and down-regulated by cysteine. The enzyme 3-hydroxybutyryl coenzyme A dehydrogenase, a key enzyme in solventogenesis in Clostridium acetobutylicum, was among the most up-regulated proteins during high toxin production. The addition of butyric acid to various growth media induced toxin production, whereas the addition of butanol had the opposite effect. The results indicate a coupling between specific metabolic processes and toxin expression in C. difficile and that certain amino acids can alter these pathways coordinately. We speculate that down-regulation of toxin production by the administration of such amino acids to the colon may become a novel approach to prophylaxis and therapy for C. difficile-associated diarrhea.
- ANSWER 14 OF 24 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
- 94344976 EMBASE AN
- DN 1994344976
- Toxin production by Clostridium difficile in a defined medium with limited TΤ amino acids.
- Yamakawa K.; Kamiya S.; Meng X.Q.; Karasawa T.; Nakamura S. AU
- Department of Bacteriology, School of Medicine, Kanazawa University, 13-1 CS Takara-machi, Kanazawa, Ishikawa 920, Japan
- Journal of Medical Microbiology, (1994) 41/5 (319-323). SO ISSN: 0022-2615 CODEN: JMMIAV
- CY United Kingdom
- DTJournal; Article
- Microbiology FS
- LΑ English
- SLEnglish
- Basal defined medium (BDM) containing vitamins, minerals and seven amino AB acids - (/L) tryptophan 0.1 g, methionine 0.2 g, valine 0.3 g, isoleucine 0.3 g, proline 0.3 g, leucine 0.4 g and cysteine 0.5 g - which appeared to be essential for good growth of Clostridium difficile was prepared. Addition of glycine 0.2 g/L and threonine 0.4 g/L to BDM

produced better growth of strain VPI 10463, and this defined medium was designated minimum amino acid-defined medium (MADM). Production of toxins A and B by strain VPI 10463 in 6 x MADM containing (/L) tryptophan 0.6 g, methionine 1.2 g, valine 1.8 g, isoleucine 1.8 g, proline 1.8 g, leucine 2.4 g, cysteine 0.5 g, glycine 0.2 g and threonine 0.4 g, was much greater than in MADM. Toxin production by 20 C. difficile strains was examined in two defined media - 6 x MADM and complete amino acid-defined medium (CADM) containing 18 amino acids and one complex medium, modified brain heart infusion medium (m-BHI). Simultaneous production of toxins A and B by all test strains was demonstrated in m-BHI and the two defined media. It was also shown that 6 x MADM was generally better than CADM and as effective as m-BHI for stimulating toxin production by 13 strains. This defined medium would be useful for studies on the physiology, metabolism and pathogenicity of C. difficile.

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NSWER 18 OF 24 CAPLUS COPYRIGHT 2004 ACS on STN
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AN 1987:421983 CAPLUS

DN 107:21983

TI Large-scale cultivation of Bordetella pertussis for production of pertussis toxin

IN Sekura, R. D.

PA United States Dept. of Health and Human Services, USA

SO U. S. Pat. Appl., 17 pp. Avail. NTIS Order No. PAT-APPL-6-889 621. CODEN: XAXXAV

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
ΡI	US 889621	A0	19861205	US 1986-889621	19860728
	US 5338670	A	19940816	US 1992-989908	19921211
PRAI	US 1986-889621		19860728		
	US 1989-338459		19890417		
	US 1990-504022		19900404	•	

B. pertussis Is cultivated and production of its toxin enhanced by

(1) incorporation of an antifoam agent; (2) controlling aeration by using
pure O or O-enriched air; and (3) regulation of Fe content in the medium.

Thus, the microorganism was precultured in a medium containing 10 mg FeSO4/L
and then cultured in a medium (pH 7.4) containing Na glutamate 965, proline
21.6 g, anti-foam C 45 mL and salts (supplemented with cystine
4.0, ascorbic acid 2.0, niacin 0.4, and reduced glutathione 10 g) at
36° with agitation. O concentration was maintained at 40% of saturation
Bacterial growth and pertussis toxin production reached a
maximum in .apprx.20 h. The maximum toxin value was approx. 6 mg/L.

- L4 ANSWER 24 OF 24 CAPLUS COPYRIGHT 2004 ACS on STN
- AN 1961:119606 CAPLUS
- DN 55:119606
- OREF 55:22546d-h
- TI Detoxification function of depot iron. II. Animal experimental studies on the detoxification of tetanus toxin by hemosiderin and reducing substances
- AU Heilmeyer, L.; Wohler, F.
- CS Med. Univ.-Klinik, Freiburg i. B., Germany
- SO Klinische Wochenschrift (1961), 39, 563-81
 - CODEN: KLWOAZ; ISSN: 0023-2173
- DT Journal
- LA Unavailable
- AB cf. CA 55, 8591e. Incubation of a mouse lethal dose of tetanus toxin with 2 mg. hemosiderin for 6 hrs. at pH 5.5 and 37°

completely protected the animals against the toxin. The toxin was also inactivated when it was incubated with hemosiderin plus ascorbic acid or cysteine or with FeSO4 or FeCl3. Incubation with distilled water did not appreciably affect activity of the toxin and incubation with ferritin only slightly prolonged survival time. In Fe-depleted mice the survival time after injection of toxin was significantly diminished. Brief incubation (10-60 min.) of toxin with ascorbic acid, FeSO4, or Reducdyn (preparation of DL-homocysteine thiolactone, L-cysteine, and fructose) produced inactivation but shorter times were not effective. Similar results were obtained in rats and rabbits. FeSO4, and to a lesser extent the other 2 compds., increased the survival time of mice when it was injected intraperitoneally as the toxin was injected subcutaneously. When these compds. were injected 30 min. later than the toxin, they afforded no protection. Oral doses of FeSO4 also partially protected against subsequently injected toxin. These and earlier results indicate that Fe ions released by reducing substances from Fe deposits, particularly in reticuloendothelial cells in sites of inflammation, may function in the inactivation of bacterial toxins.

L12 ANSWER 6 OF 19 MEDLINE on STN DUPLICATE 2

AN 2000233619 MEDLINE

DN PubMed ID: 10770786

TI Antibacterial agents and release of periplasmic pertussis toxin from Bordetella pertussis.

AU Craig-Mylius K A; Weiss A A

CS Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, Cincinnati, Ohio 45267-0524, USA.

NC RO1 AI23695 (NIAID)

SO Antimicrobial agents and chemotherapy, (2000 May) 44 (5) 1383-6. Journal code: 0315061. ISSN: 0066-4804.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200005

ED Entered STN: 20000525 Last Updated on STN: 20021218

Entered Medline: 20000518

AB Pertussis toxin accumulates in the periplasm of Bordetella pertussis prior to secretion, and we examined its fate following treatment with antimicrobial agents. Both antibiotics that inhibit protein synthesis (erythromycin and chloramphenicol), transcription (rifampin), or cell wall biosynthesis (cefoperazone and piperacillin) and magnesium sulfate (which inhibits transcription of pertussis toxin, but not bacterial growth) did not prevent release of preformed toxin. In contrast, agents that affect bacterial membranes, such as polymyxin B, lidocaine, procaine, and ethanol, inhibited release of preformed pertussis toxin. These results suggest new protein

synthesis is not required for pertussis toxin secretion, but a

functional membrane complex is required.